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The overall is to determine the 3D structures of the S. aureus enterotoxins by X-ray diffraction. A 3.5A maps of SEB has been prepared, and the tracing of the main chain is in progress.

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FOREWORD

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Martin Saf 7/3/90

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INTRODUCTION:

Progress in understanding the biological activities of the diarrheal causing staphylococcal enterotoxins (SEs) at the molecular level now has reached the point where detailed knowledge of their 3D structures is required. Much biochemical data has been accumulated about the SEs, including the amino sequences of several of them (1). A hotly pursued goal in this field presently is to explain the immunogenic activity of the SEs, in particular, their powerful stimulation of T cell proliferation (2). The first step in this process involves the formation of a binary complex between these SE and a MHC class II molecule. The latter is situated on the surface of the accessory cell, whose function is to present the binary complex to a V-beta receptor on the T cell surface, where a ternary complex consisting of the binary complex and the V-beta molecule, then forms. T-cell proliferation ensues. The process does not occur without the prior formation of the binary complex. Relevant questions about this process remain to be answered at the molecular level. Which groups on the enterotoxin molecule make binding contacts with the MHC-II molecule? Which groups interact with the V-beta receptor? What molecule structural features of the enterotoxin molecule are crucial for T cell stimulatory activity? A combination of mitagenetic and crystallographic studies relating activity on 3D structure is the most obvious approach to solving this probelm. In addition the 3D structure is vitally important for interpreting other biochemical data at the molecular level (1).

BODY:

At first we tried the method of single isomorphous replacement (SIR) to the derivation of structure factor phases for use in the preparation of a 3D map of the SEB molecule. Combining the SIR and anomolous data with solvent leveling yielded a 4A map which showed the molecular boundary, but efforts to trace the main chain were fruitless even after the phases were extended to 3.5A. An MIR (multiple isomorphous replacement) map next was constructed with data from seven heavy atom derivatives. Anomalous data from two of the derivatives also were included. The resolution of the map was 4A and the figure of merit was 0.66. Application of the solvent leveling algorithm (3) extended the resolution to 3.7A and yielded the figure of merit of 0.83. It was possible to trace approximately 75 percent of the main chain. A polyalanine model was fitted to the main chain trace on a Silicon Graphics unit. The phases were extended stepwise to 3A by repeated application of the same method. The phase extended map brought out important new features of the protein molecule. The helical regions were defined better, and the main chain certainly was altered in at least one place. Next a combined phase difference map (4) was prepared in an attempt to bring out new and improved structural information.

Our plan is to complete the tracing of the main chain and to fit the side chains. This will be accomplished by utilizing 2Fo-Fc until a completed model is obtained. The derived model will be refined by restrained least squares.

CONCLUSIONS:

Any conclusions about the 3D structure of SEB at this point must be regarded as being highly tentative since the structure determination is only partially completed. What we see presently is that there are three distinct domains of varying size in the SEB molecule, that the percentage of alpha helical regions is small, that the beta strands are considerably more prevalent than the helices, and that a large fraction of the molecules consist of a periodic secondary structural feature.

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